Fungal Conversion of Canola for Polyunsaturated Fatty Acids-added Lipids

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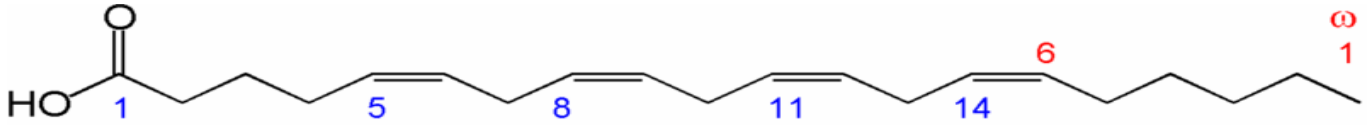
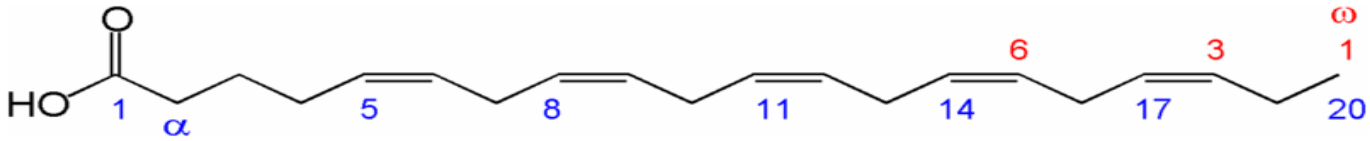
Introduction

Polyunsaturated fatty acids (PUFAs), e.g. ARA and EPA.



ARA---arachidonic acid (C20:4 ω-6)



EPA---eicosapentaenoic acid (C20:5 ω-3)

Microbial sources are commercially available (e.g. infantry formula).

In general, plant and animal lipids do not contain long-chain PUFAs (C>18).

Objectives

Determination of the feasibility for addition of PUFAs to canola oil by the fermentation of fungi using canola material as substrate.

Material and Methods

Appearance of canola materials and oil content

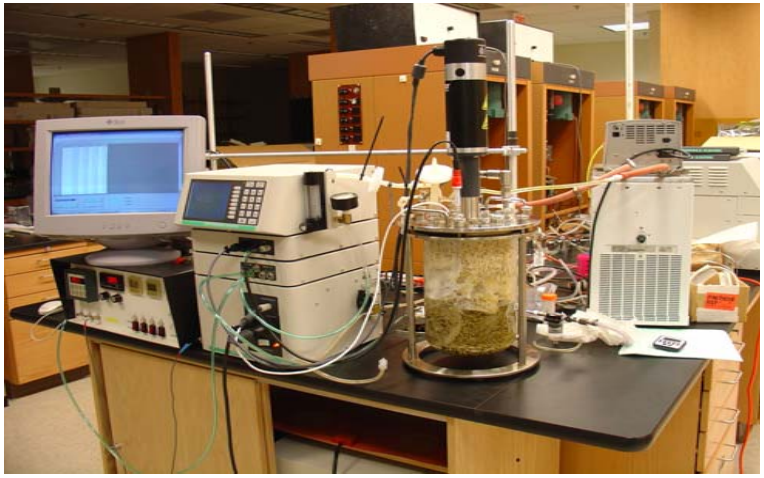
Flake	Cake	Meal
41 %, Oil (w/w)	16%	<1%

Chemical composition (%) of canola cultivars (Sosulski and Sosulski, 1990)

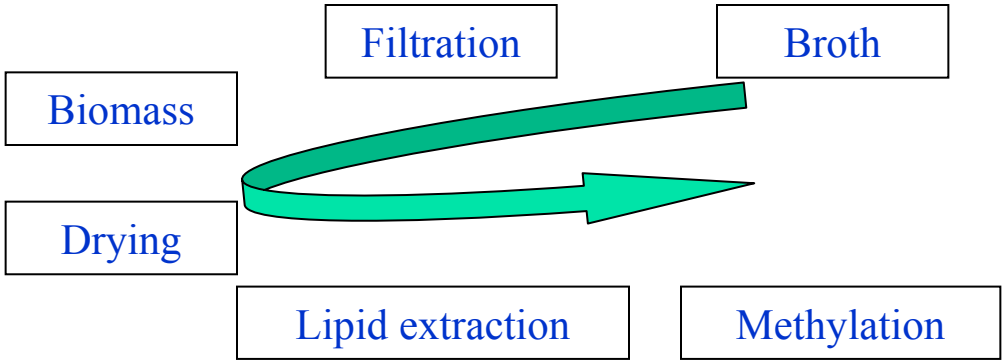
Oil and meal constituents	Brassia napus		Brassia campestris
	Westar	Regent	Tobin
Seed oil	43.1	42.7	42.2
Meal-protein	42.7	44.5	43.2
Meal-hemicelluloses	6.4	5.5	6.4
Meal-cellulose	7.2	10.2	12.1
Meal-lignin	10.7	9.8	5.6
Meal-ash	5.9	4.0	4.1

Mineral content of canola meal

Mineral	Average
Calcium (%)	0.63
Phosphorus (%)	1.08
Available P (%)	0.3-0.5**
Sodium (%)	0.10
Chlorine (%)	0.10
Potassium (%)	1.22
Sulphur (%)	0.85
Magnesium (%)	0.54
Copper (mg/kg)	5.8
Iron (mg/kg)	166
Manganese (mg/kg)	52
Molybdenum (mg/kg)	1.4
Zinc (mg/kg)	58
Selenium (mg/kg)	1.1

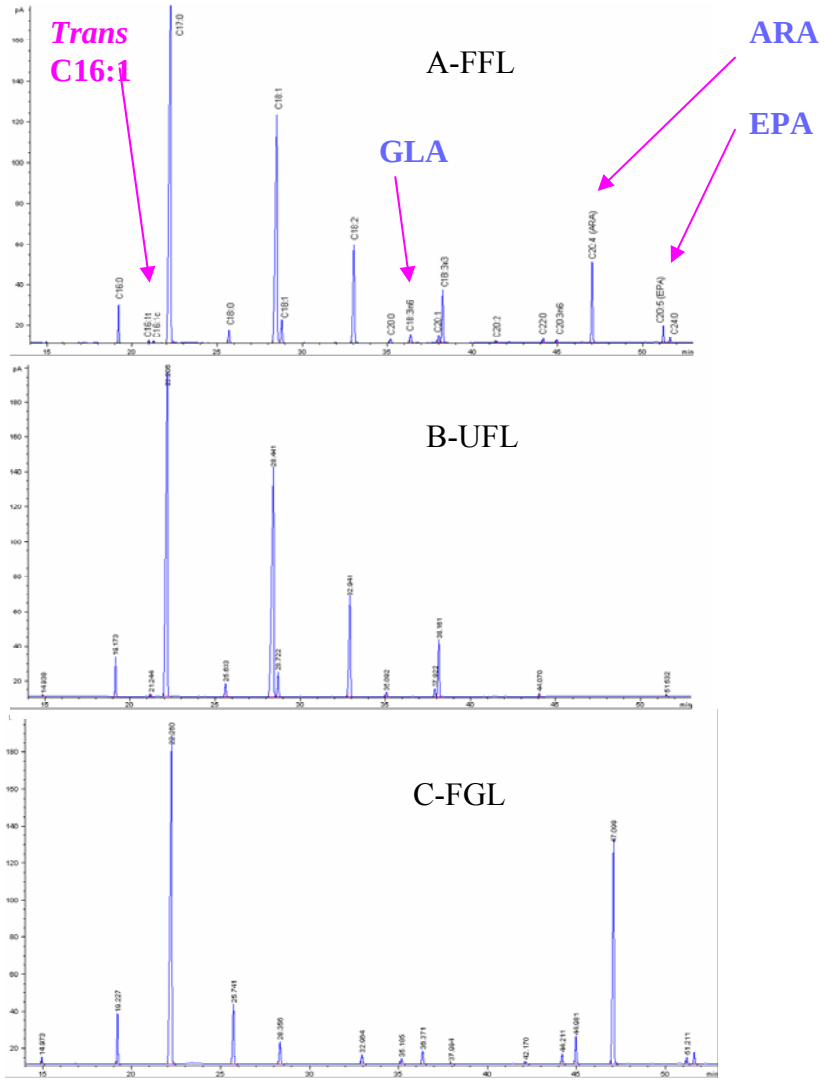


Fungus: *Mortierella alpina* (20ºC 7 days)



GC system

Results and Discussion



Four new fatty acids were added:

ARA

EPA

GLA (γ-C18:3ω6)

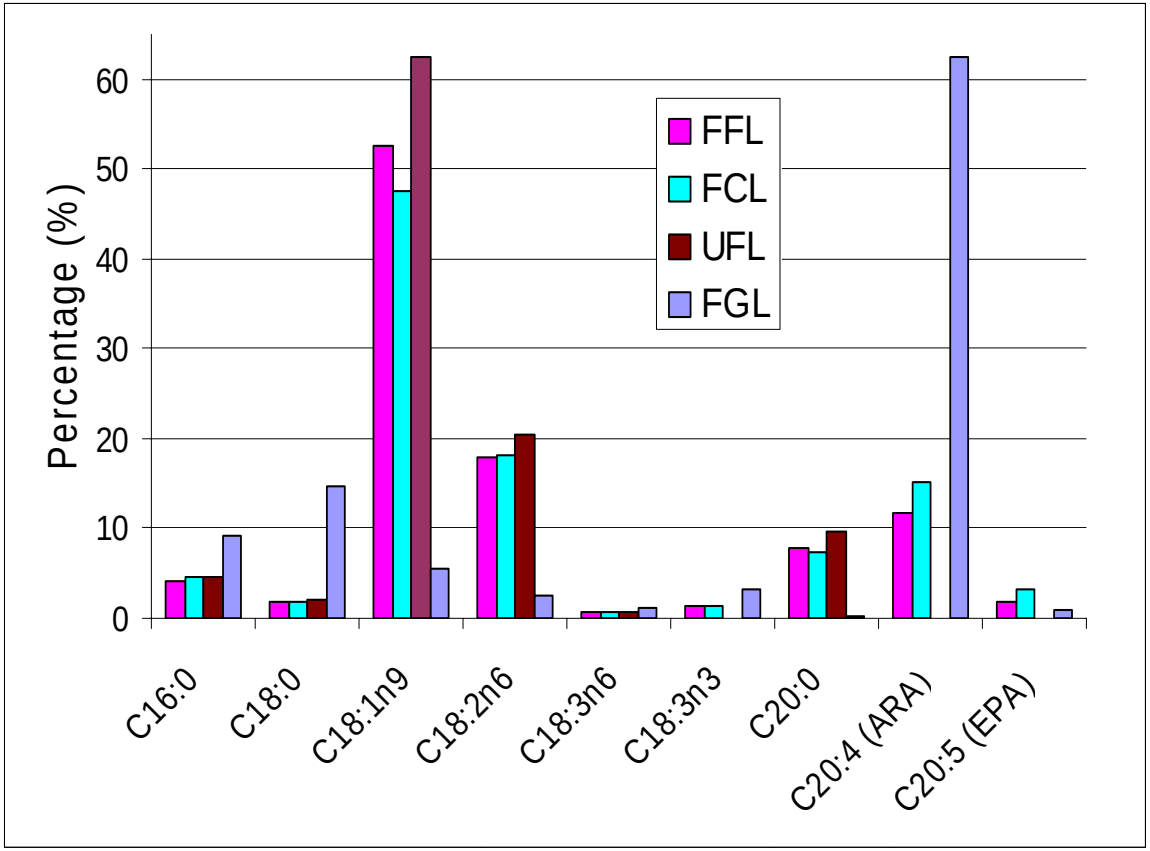
Trans C16:1 (0.2%)

FFL-Fermented flake lipid

UFL-Unfermented flake lipids

FGL-Fermented glucose lipids

Lipid profiles produced using different substrates



Production using different canola substrates and two strains in mixed culture*

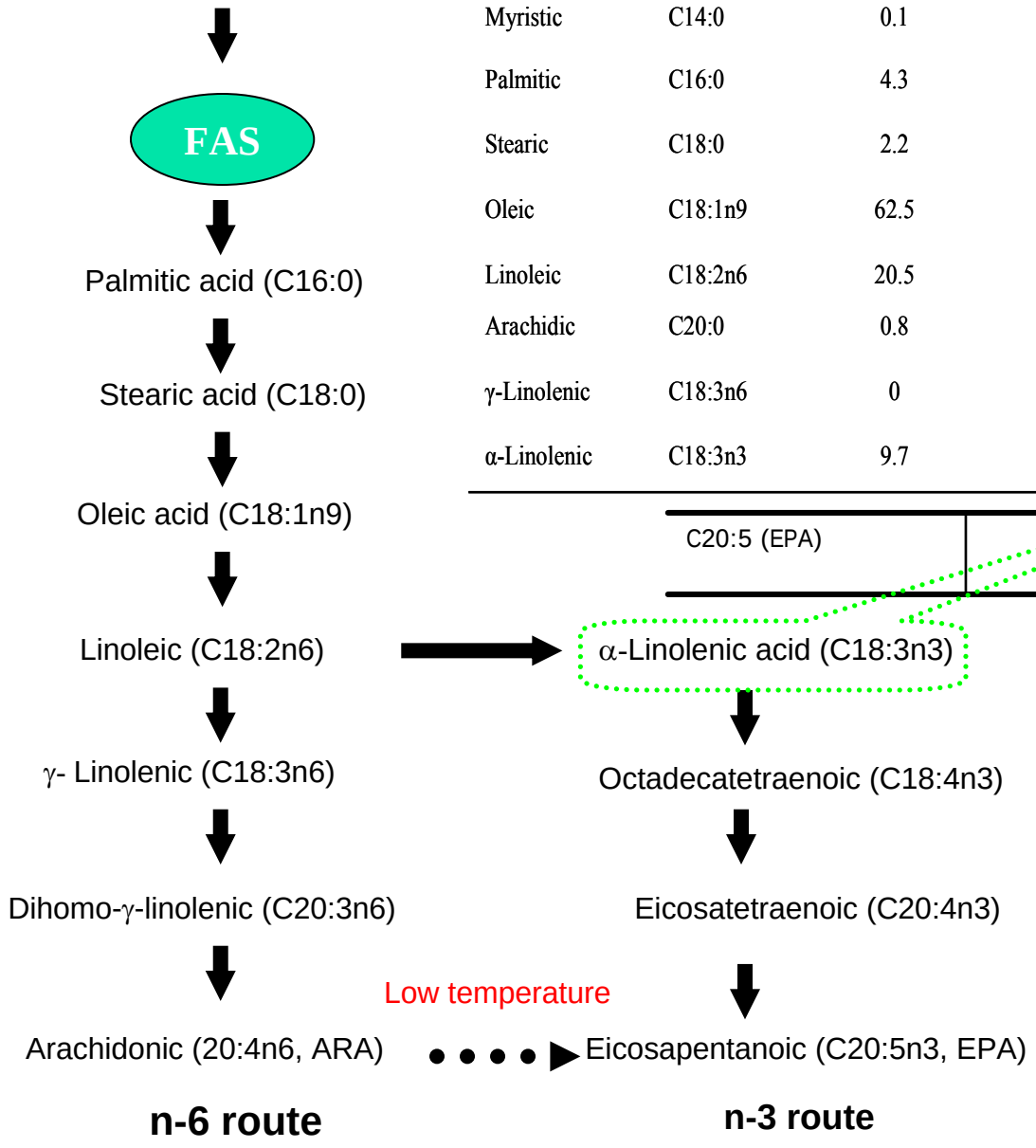
	Mortierella alpina			*Pythium irregulare*			*M.alpina* plus *P. irregulare*			Without fungi	
	Flake	Cake	Glucose	Flake	Cake	Glucose	Flake	Cake	Glucose	Flake	Cake
Total lipids (mg)	380.4[a]	179.3b	93.9[c]	439.3	186.0	105.8	416.6[d]	152.3[d]	113.1[e]	525.4	238.6
C20:4 (ARA)**	20.3 [a]	12.5 [b]	20.1[a]	1.3	1.1	2.1	26.3 [c]	22.5 [d]	37.7 [e]	None	None
C20:5 (EPA)**	3.3 [a]	2.7 [b]	0.3 [c]	2.1	1.7	3.1	4.3 [d]	3.7 [d]	0.3 [c]	None	None
P/S***	6.2[a]	6.3[a]	2.8[b]	5.4	5.5	1.1	6.5[a]	8.7[c]	2.9[d]	4.0	4.1
ARA/EPA	6.2[a]	4.6[a]	67[b]	0.6	0.6	0.7	6.1[a]	6.1[a]	125.6[c]	None	None

*Values are the means of triplicate that varied by less than 5%; The results were from Experiment 1 and 2; Different letter means statistically significant ($p<0.05$) according to LSD test; The values for strain, *Mortierella alpina,* were not statistically compared due to their obvious differences from others; **Values are mg/g substrate, based on each of 1.5 g substrate; ***P/S means the ratio of polyunsaturated over saturated fatty acids.

Comparisons of production of PUFAs using different substrates and oils*

	Flake**	Cake**	Oil-freed flake + Canola oil	Meal + Canola oil	Meal + Soybean oil	Meal + Corn oil	Meal + Olive oil
Dry biomass (mg)	1156	1097	1212	1133	1156	1165	1153
Total lipids (mg)	437.4	183.3	405.6	371.2	404.6	385.6	402.9
C20:4 (ARA)***	25.9 ^{a}	12.3 ^{b}	33.2 ^{c}	41.7 $^{d\,A}$	39.7 ^{B}	47.3 ^{C}	48.9 ^{C}
C20:5 (EPA)***	3.9 ^{a}	2.3 ^{b}	4.9 ^{c}	5.7 $^{d\,A}$	3.4 ^{B}	1.1 ^{C}	1.2 ^{C}
ARA/EPA	6.6 ^{a}	5.3 ^{a}	6.8 ^{a}	7.3 ^{A}	11.7 ^{B}	43.0 ^{C}	40.8 ^{C}

*Values are the means of duplicate varied by less than 5% from Experiment 3 using single strain, *Mortierella alpina*; **Different values compared to those in Table 2 using the same substrate were because of different batch material and culture; *** Values are mg/g substrate based on 1.5 g substrate. Different letters mean statistically significant ($p<0.05$) according to LSD test.



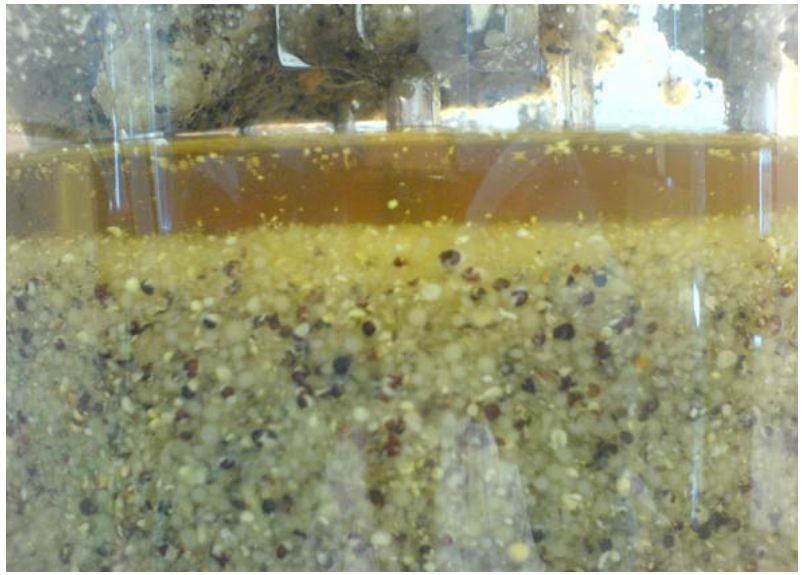
Common name	Symbol	Canola oil **		Soybean oil	Corn oil	Olive oil
		SFE	Hexane			
Myristic	C14:0	0.1	0.1	0.4	0.3	0.2
Palmitic	C16:0	4.3	4.8	10.7	10.5	11.1
Stearic	C18:0	2.2	2.1	4.8	2.0	3.7
Oleic	C18:1n9	62.5	61.5	23.0	28.9	77.7
Linoleic	C18:2n6	20.5	21.1	53.6	56.8	6.2
Arachidic	C20:0	0.8	0.7	0.4	0.4	0.4
γ-Linolenic	C18:3n6	0	0	0.3	0	0
α-Linolenic	C18:3n3	9.7	9.7	6. 9	1.1	0.7
C20:5 (EPA)		5.7		3.4	1.1	1.2

Metabolic pathways for the biosynthesis of ARA and EPA (Ratledge, 2004)

Scale-up production in 7-liter fermenter

Exp 1

Exp 2



Exp 2

Exp 3

Exp 3

Yields of ARA and EPA in fermenter and flask cultures.

	Total oil yield (g/l)	ARA		EPA	
		(mg/l)	(mg/g substrate)	(mg/l)	(mg/g substrate)
Experiment 1	7.1	444.8	19.8	66.9	3.0
Experiment 2	10.9	346.3	11.5	55.4	1.9
Experiment 3	11.7	2,900	82.8	12.6	0.4
Flask (flake)*	7.6	608.6	20.3	98.9	3.3
Flask (glucose)*	1.9	603.1	20.1	9.0	0.3

Exp 1: 90 g flake in 4-liter culture volume; Exp 2: 150 g flake in 5-liter culture volume; Exp 3:120 g glucose and 20 g yeast extracts in 4-liter culture volume.

Conclusions

Feasibility of PUFAs production utilizing canola materials was demonstrated.

- ARA (445 mg/l) and EPA (67 mg/l) were produced using canola flake in 7-liter fermenter, but the yield was lower than in flask cultures.
- ARA yield was lower than using glucose and yeast extracts, but the EPA yield was higher.
- High content of α-Linolenic acid (C18:3n3) in substrate may increase the EPA yield.

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Thanks !